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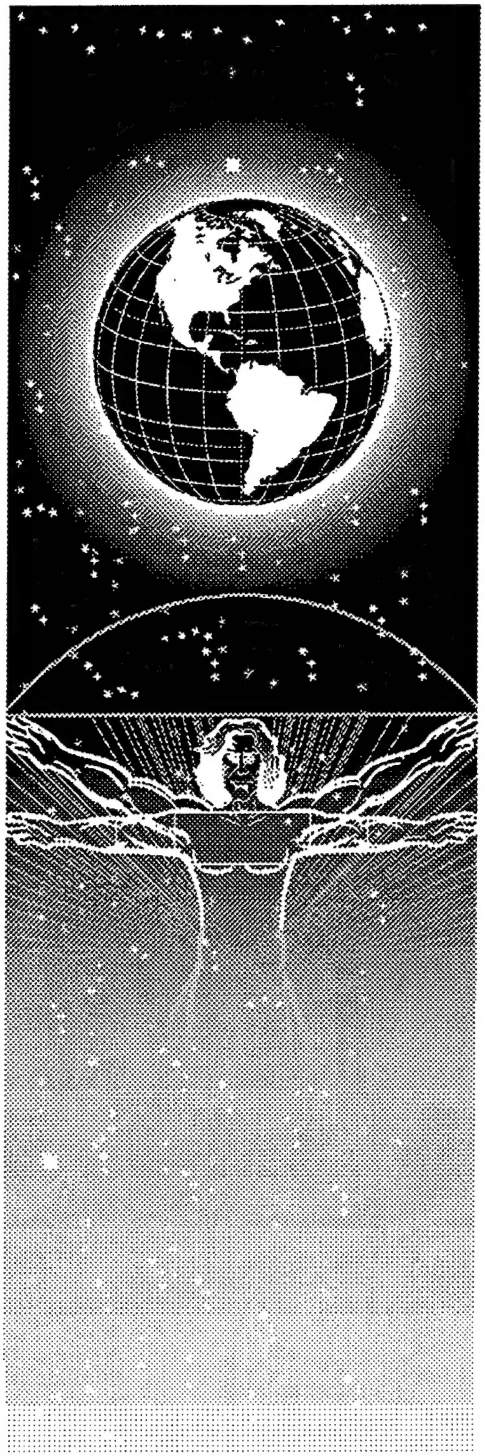

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

DCA Dosimetry: Interpreting DCA-Induced Liver Cancer Dose Response and the Potential for DCA to Contribute to TCE-Induced Liver Cancer

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The contribution of TCE-derived DCA to the induction of hepatocellular carcinoma in the mouse liver bioassay has been a pivotal issue in assessing the risk associated with oral exposure to TCE. The goal of this study was to investigate the biological plausibility of the hypothesis that TCE-derived DCA contributed significantly to TCE-induced mouse liver cancer. A pharmacokinetic analysis for DCA in naive and DCA-pretreated mice has been conducted in order to interpret the chronic bioassay liver tumorigenicity results for DCA and to evaluate the potential involvement of DCA in liver carcinogenicity induced by TCE. A physiologically motivated two-compartment model with liver and a body compartment was used to estimate the volume of distribution and the kinetic parameters for metabolic clearance by the liver. Analysis of the data confirm that there is a very large first pass metabolic clearance of DCA by the liver and that the metabolism is significantly inhibited by exposure to high doses of DCA. Most chronic drinking water studies with DCA have been conducted at concentrations of 1 to 5 g/L. At these doses inhibition of metabolism occurs rapidly relative to the length of the bioassay. Large increases in tumor incidence that have been observed at these doses correlate with increasing AUCL for the parent compound (DCA) that occurs as a consequence of saturation of its metabolism. The AUCL for TCE-derived DCA is similar to that estimated for the 0.05 g/L DCA drinking water exposure which was not associated with an increase in the prevalence of liver cancer.

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DCA DOSIMETRY: INTERPRETING DCA-INDUCED LIVER CANCER DOSE RESPONSE AND THE POTENTIAL FOR DCA TO CONTRIBUTE TO TCE-INDUCED LIVER CANCER

TECHNICAL SUMMARY

Pharmacokinetic analyses have been performed on DCA blood time course data from intravenous or oral dosing at 20 and 100 mg DCA/kg. These studies were conducted with naive mice and with mice that had been pretreated by exposure to 2g DCA/l in drinking water for 2 weeks prior to the PK evaluation. A physiologically motivated two–compartment model with liver and a body compartment was used to estimate the volume of distribution (equivalent to the volume of the body compartment) and the kinetic parameters for metabolic clearance by the liver. In naive mice the Vd, Vmax, and Km were, respectively, 0.6 l, 40 mg/hr-kg, and 0.5 mg/l. Intrinsic hepatic clearance (Vmax/Km) for a 30g mouse was 5.8 l/hr. Since liver blood flow was 0.21 l/hr, first–pass extraction by the liver will exceed 96%. Pretreatment with DCA reduced the Vmax to between 3 and 15 mg/hr-kg. For a value of 15 mg/hr-kg, intrinsic clearance becomes 2.2 l/hr with a first–pass extraction of 90%. These clearance parameters were used to predict tissue exposures to DCA and the amounts of DCA–metabolites formed in the liver of mice during chronic drinking water exposures. Because DCA is removed by efficient metabolic clearance in the liver or by much less efficient renal filtration into the urine, saturation of metabolism leads to dramatic non–linearities in the exposures of liver tissues to DCA. In the pretreated animal, saturation effects become pronounced at 1 g/l with highly non–linear increases in liver exposure predicted at concentrations of 0.5 g/l and higher. Increased incidence of tumors correlates closely with tissue exposure to DCA, and not at all well with amounts of DCA metabolized. The PK model for DCA was then coupled to a second PK model for trichloroethylene (TCE). Estimates were included to simulate the most rapid possible metabolism of TCE to DCA by assuming that all non–chlorinated metabolites from TCE were derived from DCA and that the DCA was produced directly in the initial oxidation step in TCE metabolism. Even with these worst–case assumptions, the maximum DCA produced by TCE would only be equivalent to the DCA tissue

exposures associated with 0.05 g DCA /l drinking water. Thus, the maximum tissue exposures achievable in the TCE exposure studies are well below the range of DCA tissue exposures associated with hepatic tumors. This straightforward PK analysis leads to three conclusions: (1) DCA pretreatment in mice decreased the Vmax for hepatic metabolism from 40 mg/kg/hr to less than 15 mg/kg/hr, (2) tumors arising from drinking water exposures to DCA correlate with hepatic exposures to DCA, and (3) the amounts and rates of DCA production from TCE exposures cannot be large enough for this metabolite to play any significant role in hepatic carcinogenicity with TCE. Further studies to more fully evaluate the dose response of inhibition and of pretreatment effects in rats will greatly aid in the confirmation of the conclusions reached in this report.

INTRODUCTION

Interpretation of the carcinogenicity database for dichloroacetic acid (DCA) remains an important question for trichloroethylene (TCE) risk assessment. Two questions are especially crucial. First, is DCA formed from TCE in amount sufficient to contribute to the observed liver tumor incidence from TCE? Second, if DCA levels are expected to contribute significantly, what is its mode of action? This latter question becomes important for evaluating appropriate low dose extrapolation strategies for cancer risk assessment with TCE.

In chronic drinking water studies, DCA caused liver toxicity, including cancer (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Daniel et al., 1992; Pereira, 1996; DeAngelo et al., 1997). These effects generally have been studied in mice of both sexes, but DCA also caused cancer in rat liver (DeAngelo et al., 1996). Many drinking water studies used doses in the range of 1 – 5 g/L (approximately 150 – 500 mg/kg/day for mice), while a few used lower doses but exposed animals less than two years. One two-year study had a wider dose range, including 0.05 and 0.5 g/L (approximately 8 and 80 mg/kg/day) (DeAngelo et al., 1997).

Both trichloroacetate (TCA) and DCA cause mouse liver tumors (Herren-Freund et al., 1987; Bull et al., 1990; Pereira, 1996). Either or both acids may contribute to hepatic neoplasms in mice exposed to TCE. Chloral hydrate also induces tumors in mouse liver, a response that may

also arise from, TCA and DCA (Daniel et al., 1992). However, chloral, a reactive aldehyde that spontaneously adds water to form chloral hydrate, may also contribute to its carcinogenicity. A major impediment in determining the role of these metabolites in hepatocarcinogenicity has been the limited pharmacokinetic data available for DCA. In addition, analytical chemistry problems have confused the literature on the extent of DCA production from TCE.

A study at the Division of Toxicology, WPAFB, documented that under acidic conditions TCA readily converted to DCA in oxygenated blood (Ketcha et al., 1996). The extent of the conversion decreased as the blood aged; chemical reduction of the hemoglobin to the oxygen carrying Fe^{+2} state increased the conversion. As a result of the identification of this artifact, no published data for DCA production from TCE can be accepted unequivocally, unless appropriate control experiments are included to demonstrate absence of artifactual conversion from related compounds (Ketcha et al., 1996; Merdink et al., 1998).

Studies of the pharmacokinetics of DCA have been undertaken in mice, rats, and humans (Lukas et al., 1980; Wells et al., 1980; Curry et al., 1991; Larson and Bull, 1992; Lin et al., 1993; Fox et al., 1996). These studies demonstrated that DCA was rapidly cleared from the systemic circulation by metabolism. The metabolism was cytosolic and dependent upon NADP/NADPH (Lipscomb et al., 1995). Exposure of humans to DCA decreased its subsequent metabolism upon re-exposure (Curry et al., 1991). Similar effects on DCA pharmacokinetics in mice are currently under study; some of those results are presented here (Merdink et al., 1998). In this report, pharmacokinetic data for DCA in mice obtained from studies at PNRL are used to evaluate alternate internal dose metrics in relationship to the observed cancer response with DCA.

METHODS

Pharmacokinetic Studies in Mice

B6C3F1 mice (8 wks, 23-27g, males) were exposed to DCA by i.v. injection or aqueous oral gavage (0.3 ml/25 g body weight). The mice were either naive control animals or DCA–pretreated mice. The DCA–pretreated animals received drinking water containing 2 g/L for 2 weeks. The day prior to pharmacokinetic studies, pretreated animals were provided drinking

water without DCA. During this overnight (16 h) washout period, DCA was eliminated from their bodies. Groups of control or pretreated mice then were dosed with 20 or 100 mg/kg (4 mice at 20 mg/kg, 6 mice at 100 mg/kg). Animals were bled from the tail vein at intervals (approximately 5,10, 15, 20, 25, 30, 45 minutes, 1, 1.5, 2, 3, and 4 hours); pretreated animals were monitored for a longer time than control animals due to reduced clearance in these animals.

Pharmacokinetic Analyses of DCA Exposed Animals

A pharmacokinetic model was written using Advanced Continuous Simulation Language, ACSL® (Mitchell and Gauthier Associates, Concord, MA) version 10.0. The model had liver and body compartments (Figure 1).

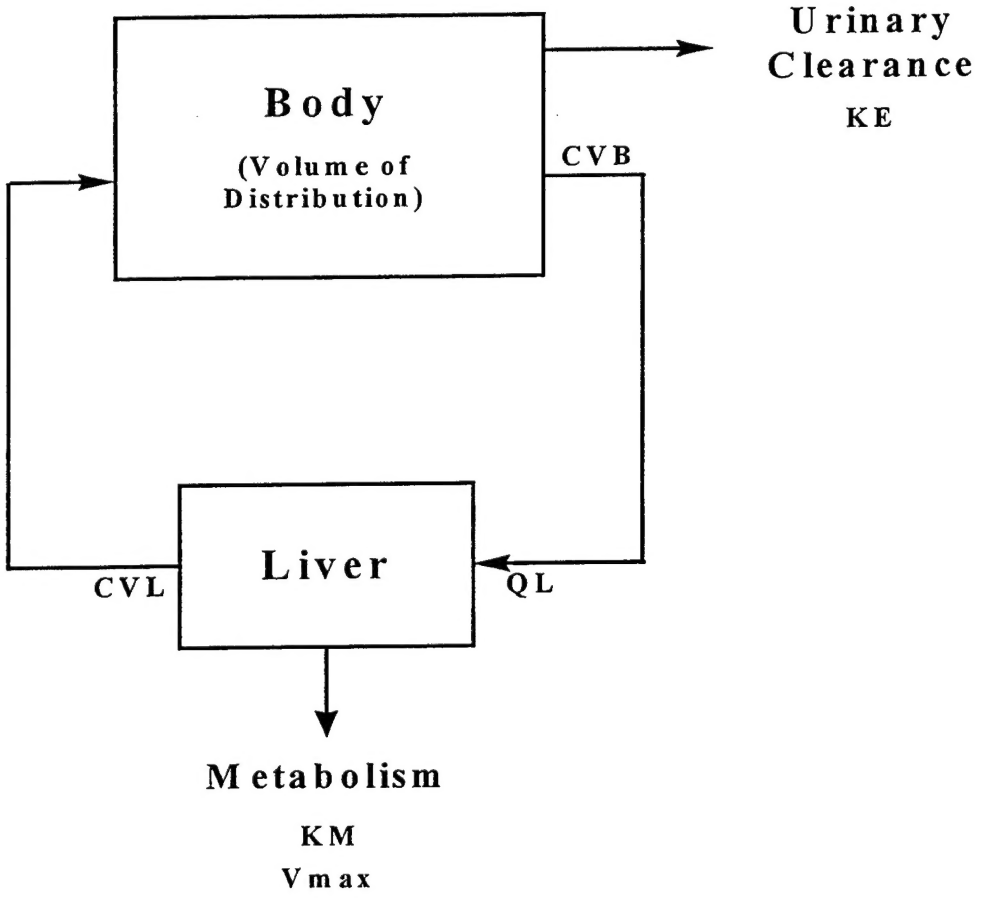

FIGURE 1: Structure of the DCA Pharmacokinetic Model. The model consists of a liver compartment with saturable metabolism and a body (volume of distribution) compartment connected by blood flow.

The body compartment represented the volume of distribution for DCA. The two compartments were described as well mixed and perfusion limited. The model included dosing by three routes, i.v. injection, oral gavage, and drinking water. Clearance of DCA by hepatic metabolism and urinary excretion were included. Parameter values are presented in Table 1.

TABLE 1: PARAMETERS FOR TWO COMPARTMENT DCA MODEL

Parameter Name	Symbol	Value	Units
Body Weight	BW	0.03	kg
Cardiac Output	QCC	15	L/hr/kg BW
Liver volume	VLC	0.05	Fraction of BW
Body volume	VBC	0.6	Fraction of BW
Liver blood flow	QLC	0.2	Fraction of QCC
Body blood flow	QBC	0.2	Fraction of QCC
Liver/blood partition	PL	1	unitless
Body/blood partition	PB	1	unitless
Metabolic rate	VMAXC	40	mg/hr/kg BW (control mice)
		8	mg/hr/kg BW (pretreated mice)
Michaelis constant	KM	0.5	mg/l
Rate of absorption	KA	.25	hr^{-1}
Rate of elimination	KE	0.003	hr^{-1}

Changes in the amount of DCA in liver are described in Equations 1–3.

$$d(\mathrm{DCA})_L/dt = \mathrm{ql} \times (\mathrm{cvb} - \mathrm{cvl}) - \mathrm{ram} + \mathrm{rao} \quad (1)$$

$$\mathrm{ram} = \mathrm{vmax} \times \mathrm{cvl} / (\mathrm{km} + \mathrm{cvl}) \quad (2)$$

$$\mathrm{cvl} = \mathrm{cl} / \mathrm{pl} \quad (3)$$

The overall change was a function of the blood flow (*ql*), the concentrations entering (*cvb*) and leaving (*cvl*) the liver, DCA absorbed from the gastrointestinal tract (*rao*), and metabolism (ram). The rate of metabolism (ram) was described as a saturable enzymatic process (Equation 2). Absorption from the gastrointestinal tract (*rao*) is described below. Finally, the venous concentration of DCA exiting the liver (*cvl*) was assumed to be equilibrated with the bulk liver concentration (*cl*), according to the partition coefficient (*pl*) describing the equilibrium liver/blood concentration ratio. Partition coefficients were given a value of 1.0 based upon limited *in vitro*

estimates (Jepson et al., 1994) and the absence of data on tissue concentrations that would justify another value.

$$d(\mathrm{DCA})_B/dt = \mathrm{qb} \times (\mathrm{cvl} - \mathrm{cvb}) - \mathrm{ke} \times \mathrm{cvb} + \mathrm{iv} \qquad (4)$$

Changes in the amount of DCA in the body compartment were described similarly (Equation 4). In addition to the DCA delivered and removed in blood, two additional pathways were modeled. Dichloroacetate was eliminated by first order clearance (*ke* × *cvb*) representing urinary clearance of the parent compound. Intravenous injection added compound directly to the compartment.

$$d(\mathrm{DCA})_{ST}/dt = \mathrm{raw} - \mathrm{ka} * \mathrm{ast} \qquad (5)$$

Dichloroacetate was added to the stomach compartment (Equation 5) in drinking water (*raw*) or as a bolus gavage dose. The rate of oral absorption of DCA from the stomach (*rao* in Equation 1) was described as a function of the amount in the stomach (*ast*) and a first order uptake rate (*ka*). For oral gavage, the dose (*odose*) was described as an instantaneous addition to the stomach by setting *odose* as the initial amount when integrating Equation 4. Exposure to drinking water was modeled using data describing the water consumed during 30 minute intervals as a percentage of total water consumption (Yuan, 1993). Total consumption was allometrically scaled as a function of $BW^{0.75}$ using the daily intake of 5 ml/25 g mouse (Yuan, 1993). Using a "Table" function provided by ACSL, drinking water intake was described as continuous addition to the stomach of DCA–containing water at a specific rate (*raw*) for each 30 minute period.

Pharmacokinetic Analyses for TCE Exposure

To estimate potential production of DCA from TCE, models for these two compounds were linked. The TCE model was derived from existing models and described oral uptake, metabolism, and exhalation of parent compound (Barton et al., 1995; Clewell et al., 1995). Oxidative metabolism of TCE in the liver was described as a saturable pathway. An upper bound on the fraction of TCE metabolism (*frac*) potentially giving rise to DCA was derived by assuming that all dechlorinated metabolites were derived from formation of DCA while total trichloro–compounds

(TTC, i.e. trichloroacetate, trichloroethanol and its glucuronide) could not be involved in DCA formation. The value of *frac* was estimated at 0.12 using the studies of Dekant et al. (1986) and Prout *et al.* (1985) (Prout et al., 1985). The TTC were 93 – 94% of urinary metabolites at three oral gavage doses (2, 20, and 200 mg/kg) in NMRI mice (Dekant et al., 1986). It was assumed that 94% of unspeciated radioactivity in feces and carcass were also TTC; thus 6% of materials in urine, feces, and carcass were estimated to be non–TTC. The total non–TTC materials were estimated as the sum of the exhaled CO_2 and the 6% of TCE–derived materials in urine, feces, and carcass. All doses had total recoveries of 97 to 99% of administered radioactivity. The estimated percent non–TTC metabolites in the Dekant *et al* (1986) dose groups were 8, 9, and 11% at 2, 20, and 200 mg/kg respectively. In B6C3F1 mice, estimated percent non–TTC metabolites were 16, 14, 12, and 12% in the 10, 500, 1000, and 2000 mg/kg dose groups respectively (Prout *et al.* 1985). The average value of these estimates for *frac* was 12%.

RESULTS AND DISCUSSION

Estimating DCA Model Parameter Values

Values for the two compartment model for DCA were estimated using blood time course data following i.v. injection and oral exposure. Two doses of DCA were used, 100 and 20 mg/kg, for a body weight averaging 25g. As illustrated in Figures 2 and 3, blood time course data from control animals were simulated reasonably well using a volume of distribution of 60% of body weight (*vbc* = 0.6) and a metabolic rate of 2.5 mg/h (*vmaxc* = 40). The elimination constant, *ke*, was set to 0.003 which eliminated virtually all of a 100 mg/kg dose within 24 hours if there were no metabolism. This choice was based on the kinetics of trichloroacetate. Using these parameter values, 8% and 2% of the dose was eliminated unmetabolized in the urine at the high and low doses, respectively, which was generally consistent with the results of oral dosing (Larson and Bull, 1992). By contrast, animals pretreated with DCA had a much lower rate of metabolism. The i.v. dose of 100 mg/kg was well fitted with a metabolic rate of 0.5 mg/h (*vmaxc* =8) (Figure 4). Using these same parameter values the fit to the blood time course for pretreated mice injected with 20 mg/kg was poor. A reasonable fit was obtained only by assuming a still lower rate of metabolism (i.e. *vmaxc* =3) and partial delivery of the intended i.v. dose (*ivdose* =15) (Figure 5).

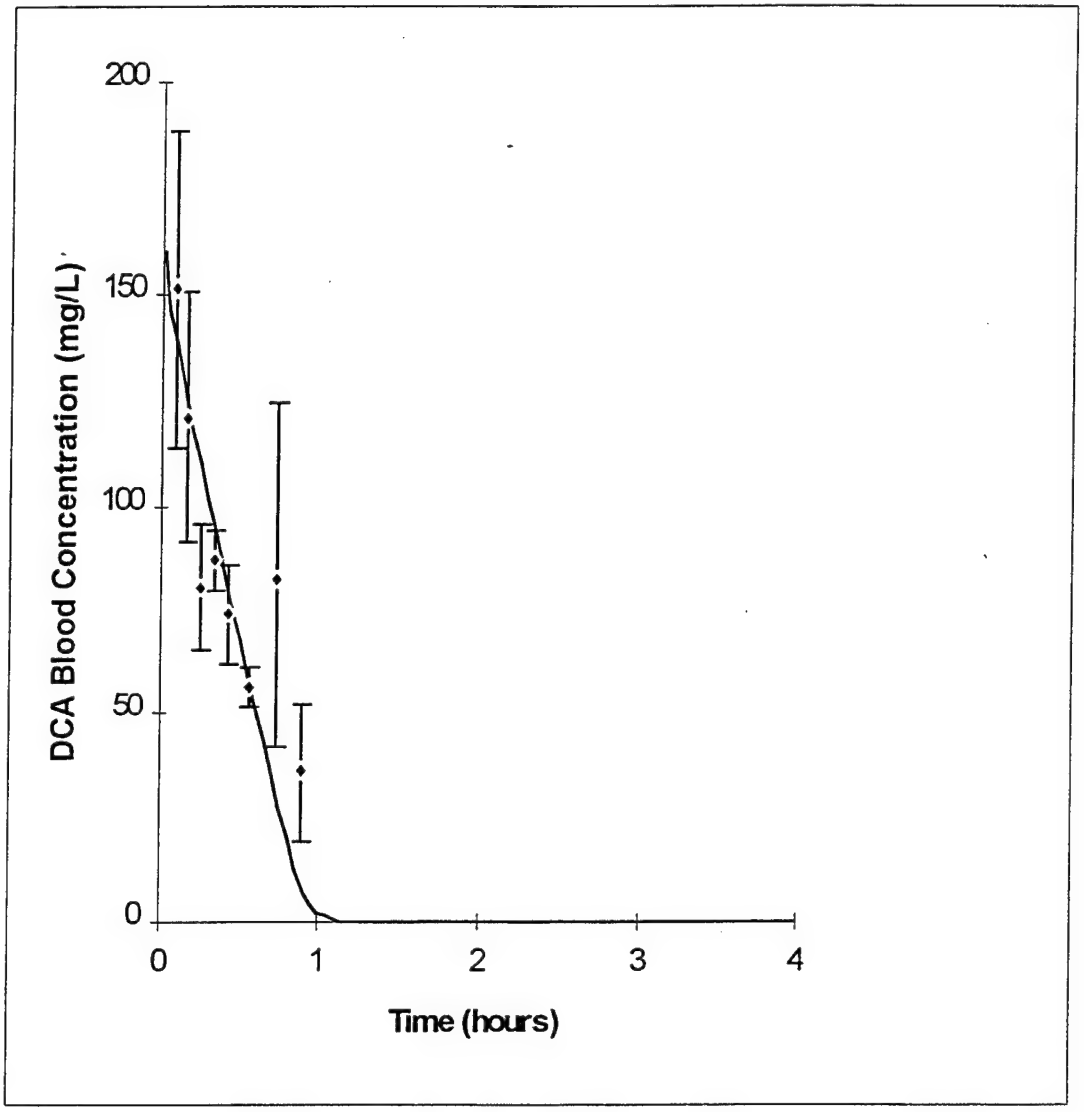

FIGURE 2: DCA blood timecourse - i.v. injection of control rats with 100 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

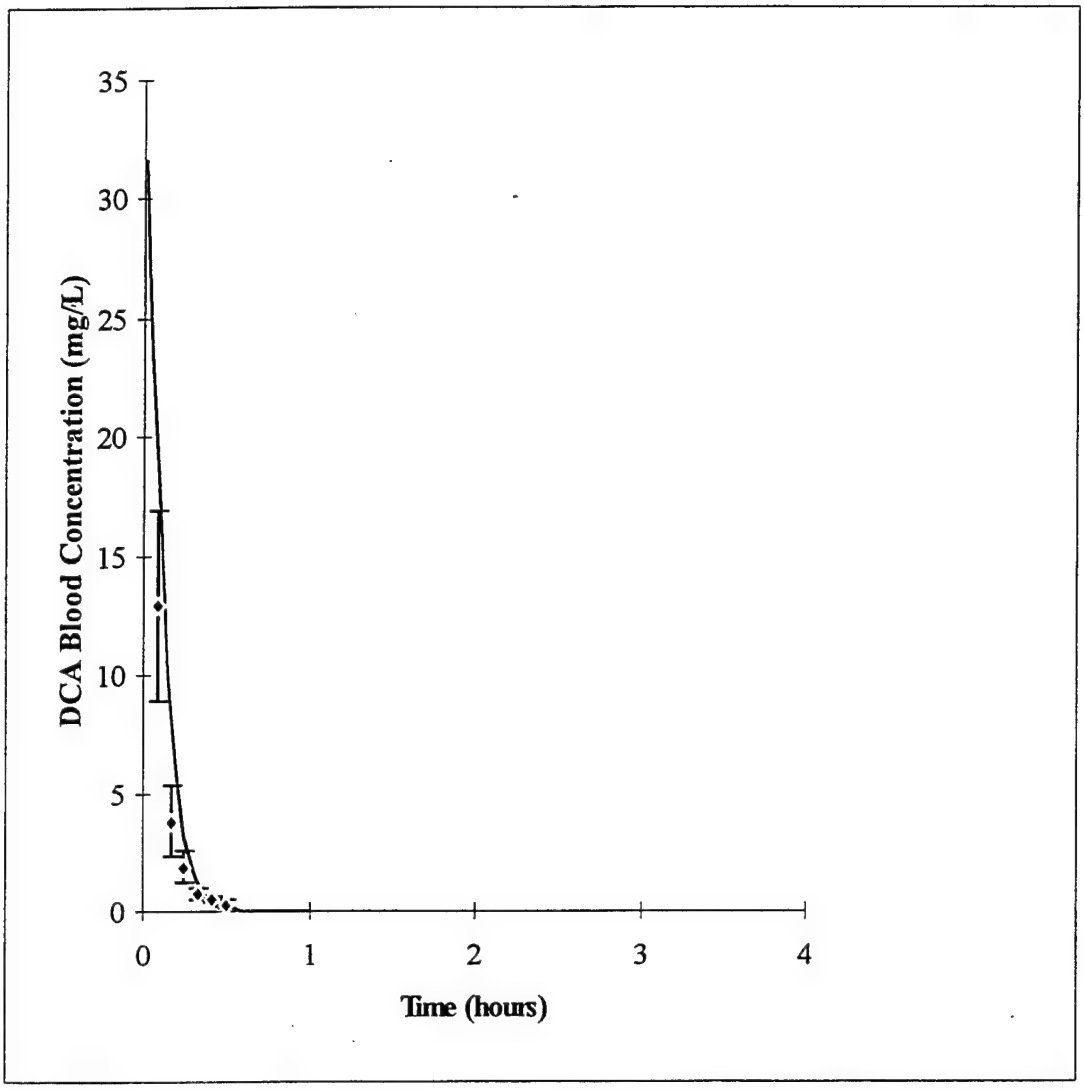

FIGURE 3: DCA blood timecourse - i.v. injection of control rats with 20 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

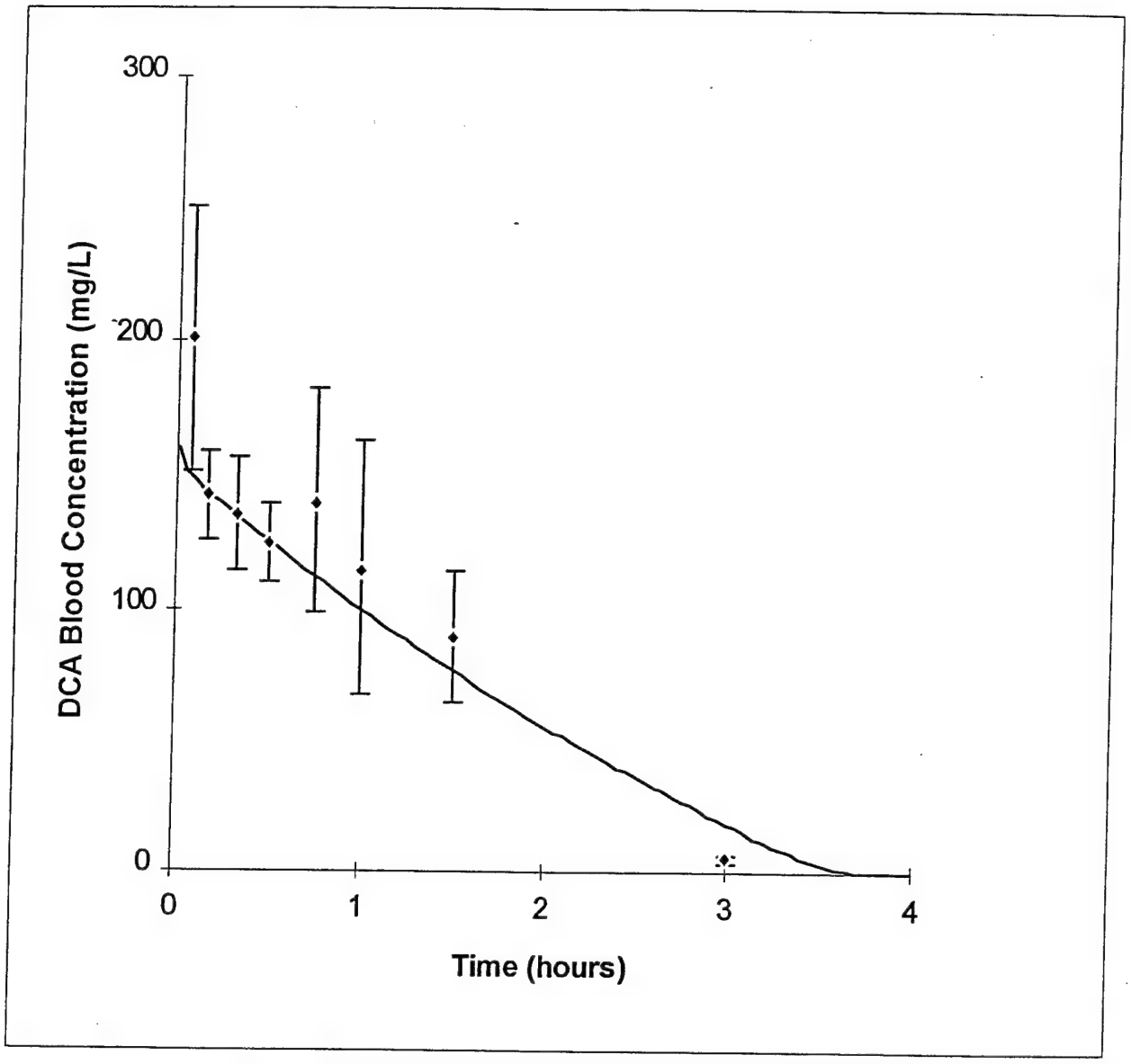

FIGURE 4: DCA blood timecourse - i.v. injection of DCA-pretreated rats with 100 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

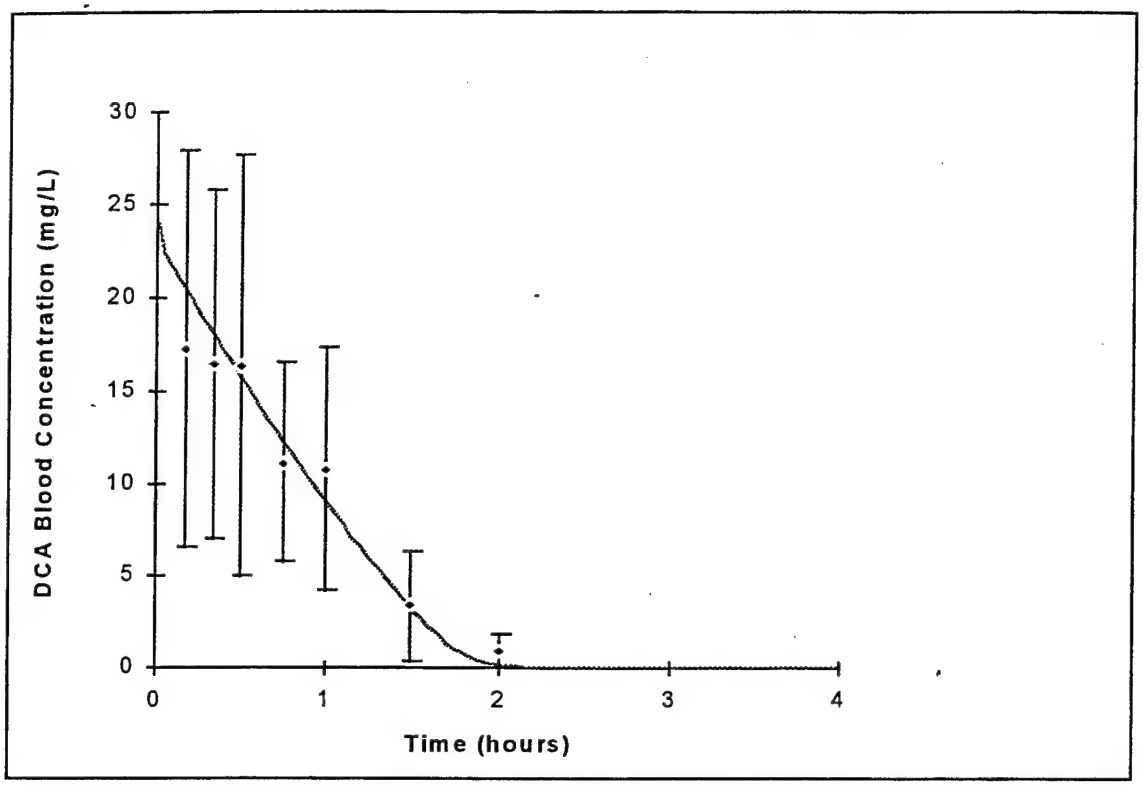

Figure 5: DCA blood timecourse - i.v. injection of DCA-pretreated rats with 20 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

Oral dosing with 100 and 20 mg/kg resulted in much lower blood levels. Previous studies had found only about 1% of an oral dose in feces indicating that the compound was well absorbed (Larson and Bull, 1992). Control animals treated with 100 mg/kg had very low blood concentrations of DCA that were below the level of detection by one hour. To fit these data, the uptake rate constant (*ka*) was fixed at 1.1 with the control metabolic rate established in the i.v. dosing studies, *vmaxc* =40 (Figure 6). The blood time course of pretreated animals given 100 mg/kg was consistent with a faster uptake rate constant, *ka* = 5, with *vmaxc* =8 (Figure 7). Finally, pretreated animals were exposed to 20 mg/kg. These data were simulated with *ka* =2.5 and a higher metabolic rate than used for the other data from pretreated animals, *vmaxc* =15 (Figure 8). These oral pharmacokinetic data were fitted using values of *ka* that varied between 1.5 and 5, so subsequent analysis used a value of 2.5 as a reasonable estimate.

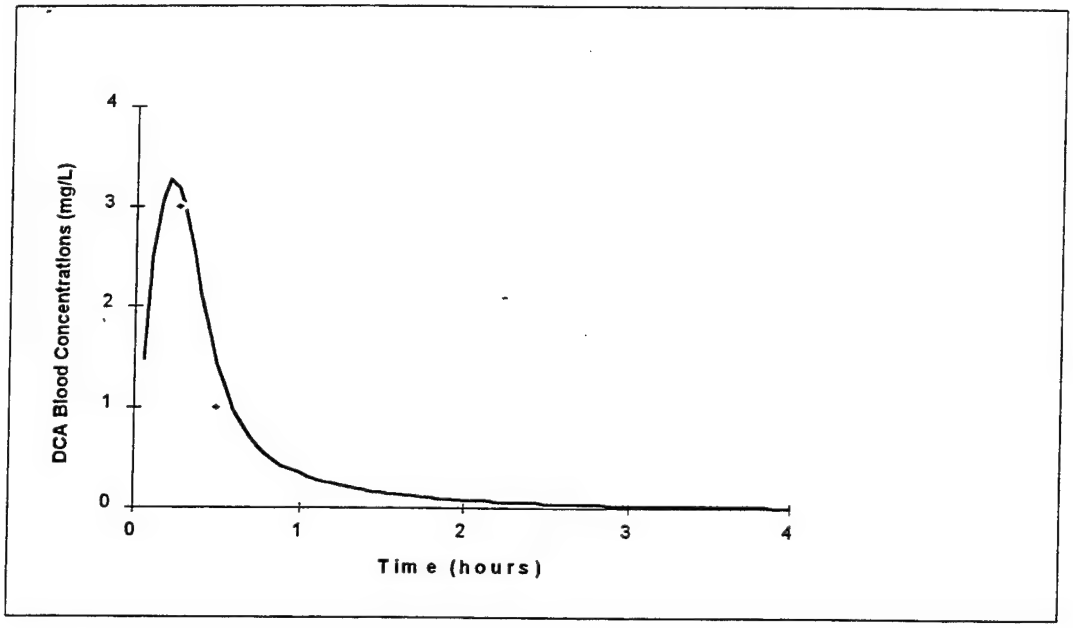

FIGURE 6: DCA blood timecourse - oral gavage of control rats with 100 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

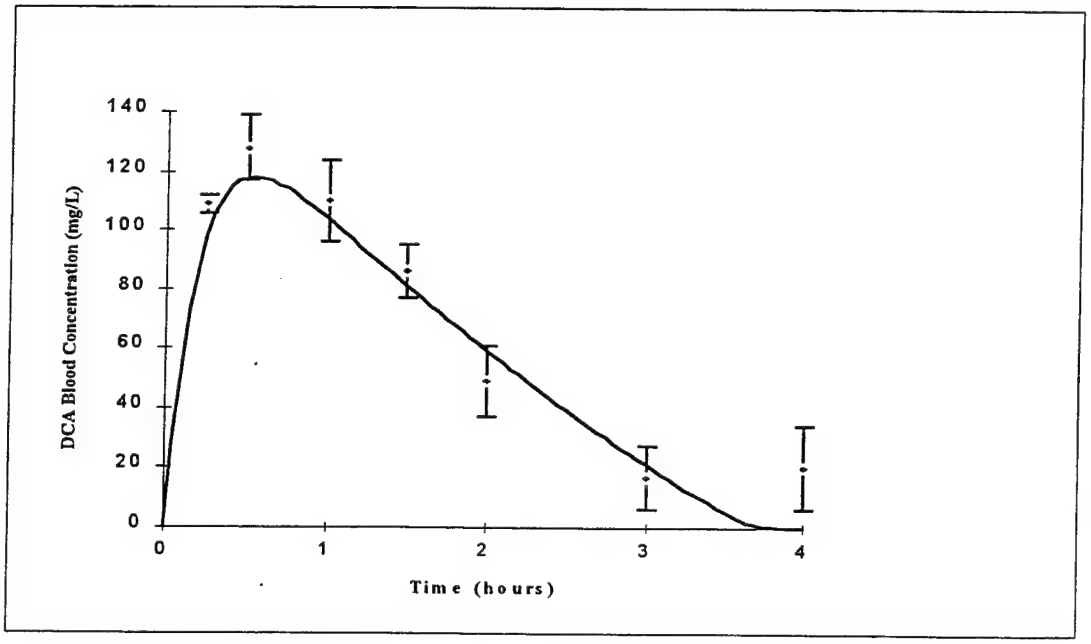

FIGURE 7: DCA blood timecourse - oral gavage of DCA-pretreated rats with 100 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

Using these i.v. and oral blood time course data, a reasonable description was obtained for the metabolism of DCA under several different conditions. Control animals cleared DCA from blood very rapidly (*vmaxc* =40), while animals pre-exposed to high DCA cleared DCA at a much slower rate (*vmaxc* = 3 to 15). Due to the large intrinsic clearance by the liver, there was a very large first pass effect when DCA was dosed orally. Given this information, the pharmacokinetics of DCA following drinking water exposure to a wide range of doses can be estimated.

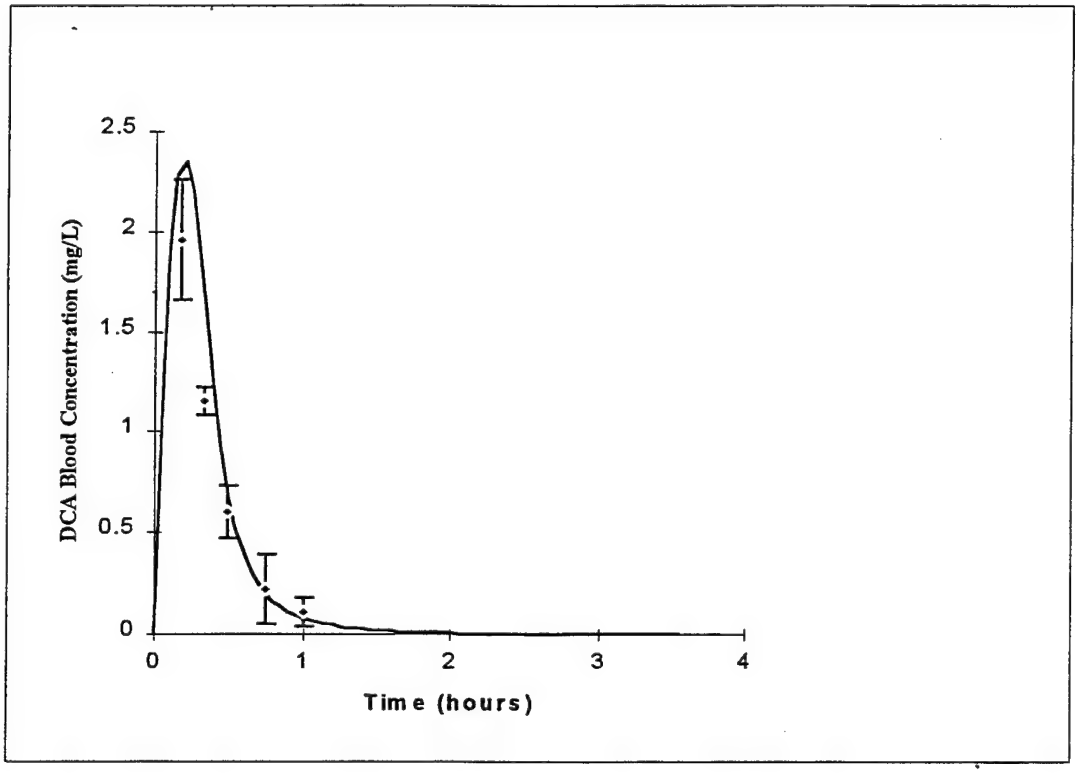

FIGURE 8: DCA blood timecourse - oral gavage of DCA-pretreated rats with 20 mg/kg. The data points and standard deviations are illustrated along with a solid line for the model simulation (*cvb*).

DCA Pharmacokinetics and Carcinogenicity Following Drinking Water Exposure

Water consumption by mice is illustrated in Figure 9. As seen in Figure 10, at relatively low DCA concentrations in control animals (e.g. 0.1 g/l, *vmaxc* = 40) the simulated body compartment concentrations simply mimicked the intake of drinking water due to its rapid absorption and

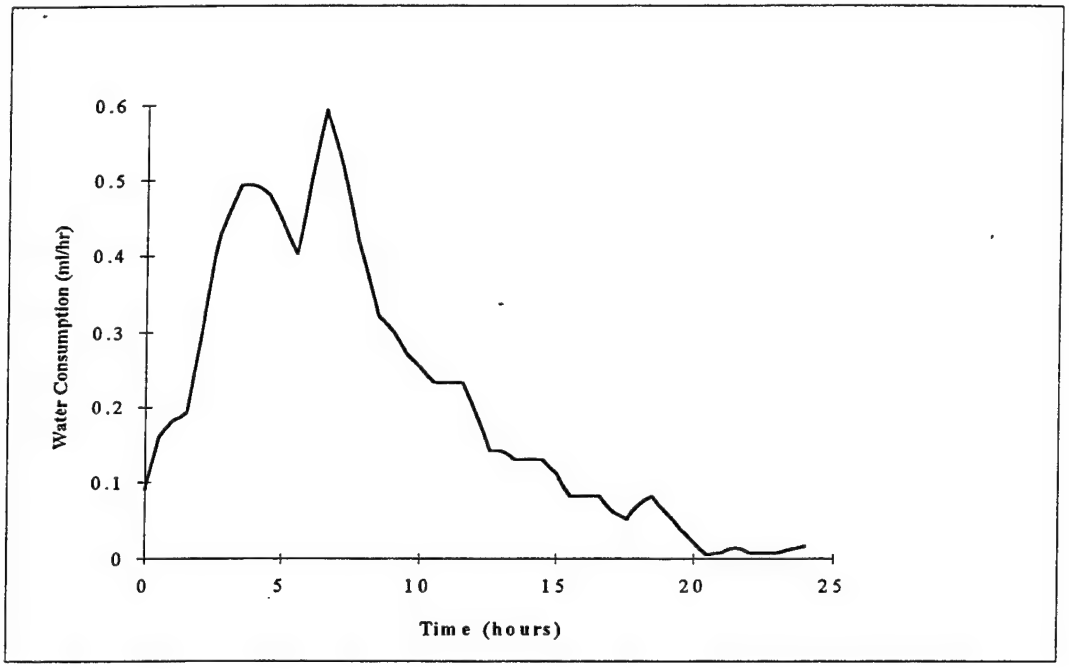

FIGURE 9: Drinking water consumption by mice. The solid line plots the drinking water consumption (*rawc*) used for simulating drinking water exposures.

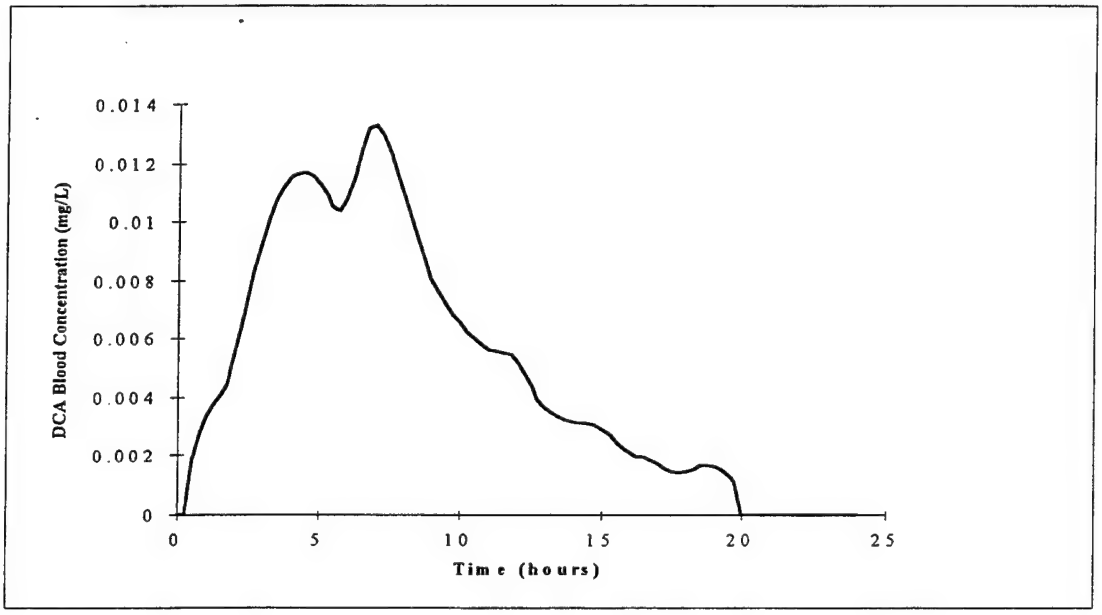

FIGURE 10: DCA blood timecourse - 0.1 g/L in drinking water. The solid line is the model simulation (*cvb*) assuming uninhibited metabolism (*vmaxc =40*).

clearance. If DCA metabolism were not inhibited at any concentration, the dose response for area under the concentration curve in liver (AUCL) would be highly nonlinear (Figure 11). At low concentrations, the increase in AUCL was proportional to the increase in DCA consumed. As metabolism saturated, increases in dose led to greater than proportional increases in AUCL because the urinary clearance was relatively small compared to the metabolic clearance. If DCA metabolism were inhibited at all doses (e.g. pretreated animals were used), the dose response curve for AUCL also would be markedly nonlinear, but the values of AUCL would be much greater (Figure 12, note difference in y–axis scale compared to Figure 11). The curves for AUCL and total amount metabolized under these different conditions are overlaid in Figure 13, showing metabolic inhibition occurred at lower doses and increased the AUCLs. The values for AUCL at the higher concentrations, when metabolism saturated, are sensitive to the estimate of *ke*. However, the urinary clearance is much slower than the metabolic clearance, so the overall shape of the curve will be similar regardless of the exact rate of urinary clearance.

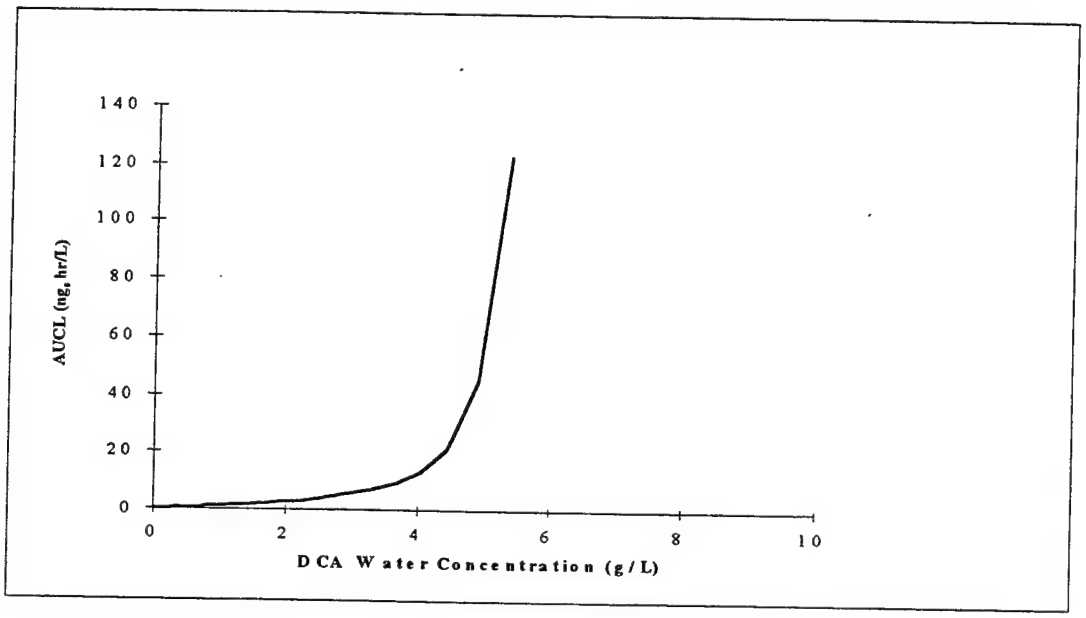

FIGURE 11: DCA in drinking water dose response for AUCL assuming uninhibited metabolism. The solid line plots model estimates for the area under the DCA concentration curve in liver (AUCL) with drinking water concentrations of DCA ranging from 0.01 to 5g/L and uninhibited metabolic capacity (*vmaxc=40*).

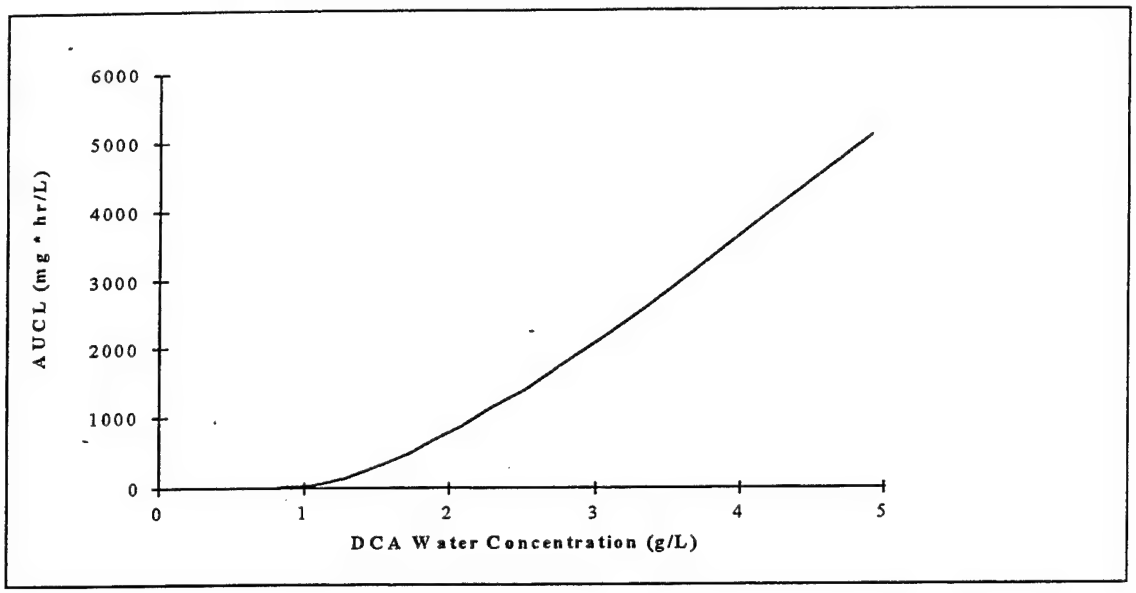

FIGURE 12: AUCL assuming inhibited metabolism of DCA - drinking water dose response. The solid line plots model estimates for the area under the DCA concentration curve in liver (AUCL) with drinking water concentrations of DCA ranging from 0.01 to 5 g/L and uninhibited metabolic capacity (*vmaxc* = 8).

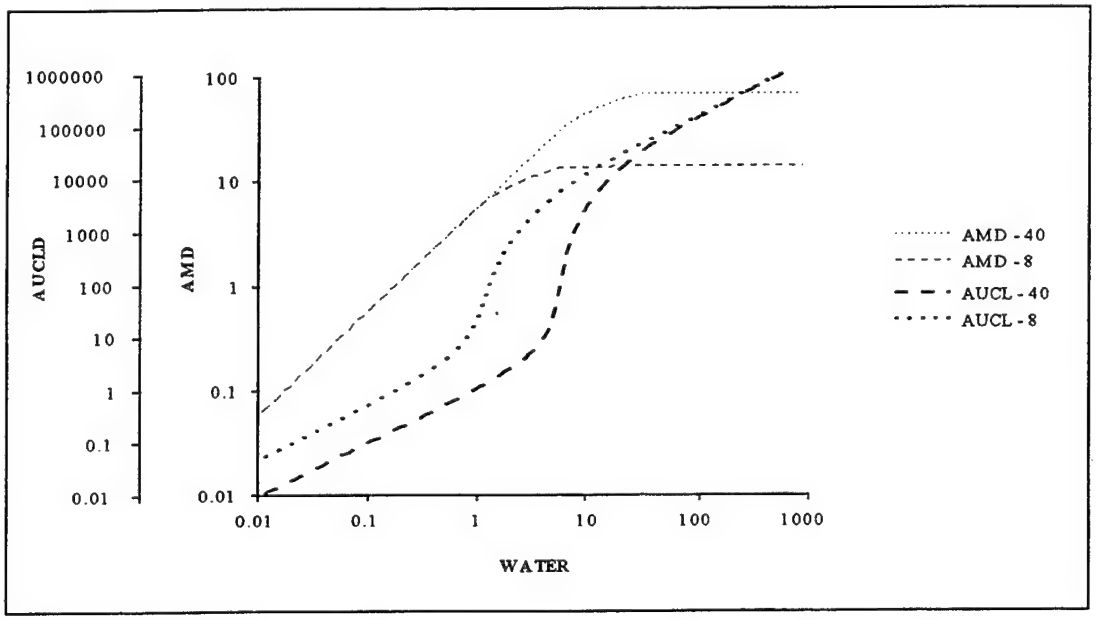

FIGURE 13: DCA AUCL and amount metabolized - drinking water dose response. AUCL values from the previous two figures are overlaid along with the total amount of DCA metabolized (*amd*). AMD-40 and AUCL-40: uninhibited metabolism, AMD-8 and AUCL-8: inhibited metabolism.

The objective of this analysis was to estimate tissue concentrations of DCA in the drinking water bioassays and how this would influence evaluation of the tumor incidences observed in those bioassays. To fully determine DCA exposures and internal dose metrics for these bioassays, data would be required on water consumption, body weight and metabolic status. Absent detailed data, estimates can be made using available information. At low concentrations, the metabolism likely would be the same as observed in control animals; at high concentrations the metabolism would be that observed in pretreated animals. The dose response for the transition to the inhibited metabolism has not been investigated. Indications of inhibition have been observed following two weeks exposure to 0.2 g/L (IS and RJB, unpublished observations). It is known to occur in humans following a single therapeutic dose, 50 mg/kg (Curry et al., 1991), and may

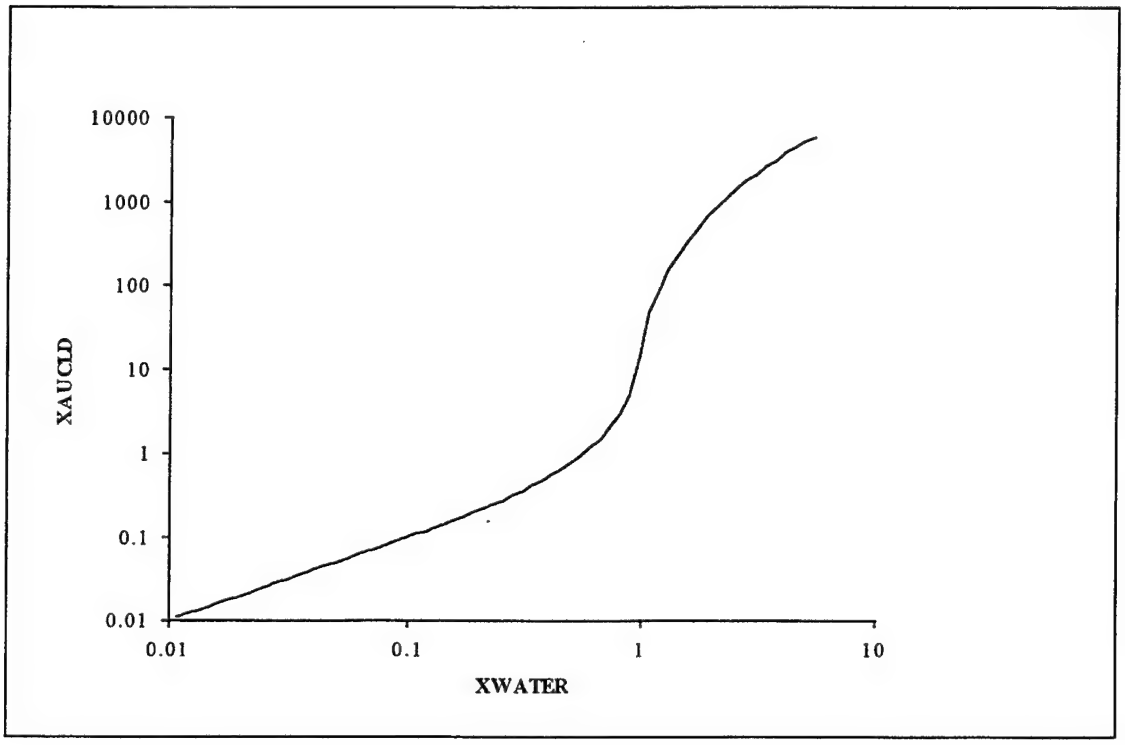

FIGURE 14: AUCL assuming a transition from uninhibited to inhibited metabolism of DCA - drinking water dose response. The solid line plots model estimates for the area under the DCA concentration curve in liver (AUCL) with drinking water concentrations of DCA ranging from 0.01 to 5 g/L. Metabolism was assumed to be uninhibited at low concentrations (*vmaxc* = 40) and became inhibited between 0.02 and 1.0 g/L.

TABLE 2: DCA DRINKING WATER EXPOSURES, CANCER RESPONSE AND SIMULATED INTERNAL DOSE METRICS

EXTERNAL DOSE METRICS		INTERNAL DOSE METRICS@		RESPONSES	
Water Concentration. (g/L)	Measured Mean Daily Doses (mg/kg/d)	AUCL (mg–hr/L)	AM (mg)	Hepatocellular Carcinoma P Prevalence (%)	Multiplicity
0	0	0	0	26	0.26
0.05	5	0.026	0.15	NS	0.58*
0.5	58	0.45	1.7	NS	0.68*
1	132	7.5	3.9	71*	1.29*
2	407	936	9.4	95*	2.47*
3.5	628	2521	11.4	100*	2.90*

NS Not statistically different from control

* Statistically different from control

@Internal dose metrics predicted by adjusting the amount of water consumed to match the measured mean daily doses assuming a 30 g mouse. Water consumption was modeled as 42, 48, 55, 85 and 75 ml/kg for the 0.05, 0.5, 1, 2 and 3.5 g/L doses

Reference: (DeAngelo et al., 1997)

well occur more rapidly than the two week period used in these studies to pretreat the animals. To illustrate what the dose response for AUCL might look like, the transition to inhibited metabolism was assumed to occur linearly over the doses of 0.02 to 1.0 g/L (Figure 14). Using this approach, the AUCL and AM predicted for the measured daily dose of a dose response chronic bioassay are presented in Table 2. (The total amount of drinking water was varied to match the measured daily doses assuming a 30 g mouse.) While there are small increases in the amount of DCA metabolized above 0.5, there is a large increase in the AUCL. Thus, tumor prevalence is closely associated with increased exposure to parent compound rather than to concentrations of metabolites.

Bounding DCA production from TCE

There continues to be controversy over the potential roles of TCA and DCA in the liver carcinogenicity of TCE. Evaluation of the role of each has been seriously impeded by the presence of an analytical artifact that produces DCA from TCA in the presence of oxygenated

hemoglobin (Ketcha et al., 1996). It is notable that in studies with adequate analytical controls to eliminate artifacts, little or no circulating DCA has been detected in mice or humans exposed to TCE or its metabolites (Ketcha et al., 1996; Merdink et al., 1998). Therefore, potential flux through a DCA forming pathway must be estimated in other ways, rather than being based upon measured blood levels. Using the DCA model developed above, estimates were made of potential DCA production from TCE, the likelihood of its detection, and it's possible involvement in TCE–induced hepatocarcinogenicity.

To maximize the likelihood that DCA would be detectable in blood it was assumed that any TCE metabolized but not accounted for by trichloro–metabolites, could have formed DCA subsequent to the initial oxidation step. This analysis ignored the glutathione transferase conjugation pathway which accounts for only a few percent of TCE metabolism. The metabolic pathways leading to metabolites lacking three chlorines have not been clearly identified. Therefore, this calculation should be considered an upper bound on DCA formation; dechlorination does not necessarily lead to DCA.

Figures 15 and 16 simulate dosing mice with 1000 or 2000 mg/kg TCE by oral gavage. Because DCA metabolism was simulated as a fraction of the total oxidative metabolism of TCE, the prolonged plateau in DCA concentration represents the balance between saturation of cytochrome P450 2E1 metabolism of TCE and the rapid metabolic clearance of DCA.
The efficiency of the liver metabolism is illustrated by the amount of DCA that was estimated to be formed in the liver from a 2000 mg/kg dose of TCE, 1.6 mg, versus the 60 μg that would reach the body (estimated by raising *ke* to 1000 so any DCA leaving the liver would be eliminated.)

The maximum concentration of DCA observed in these simulations was 0.07 mg/L; the reported limits of detection (LOD) and quantitation (LOQ) were 1.4 and 1.9 mM or 0.18 and 0.24 mg/L (Merdink et al., 1998). Therefore, detection of DCA following TCE dosing would require better analytical sensitivity. This estimate is sensitive to the *Km* estimated for the metabolism of DCA; a *Km* of 1.0 rather than 0.5 gives a DCA concentration of 0.1 mg/L. Regardless of the exact

value of this parameter, the achieved DCA concentration will be quite low. These calculations were based on an upper bound estimate of DCA production from TCE; actual values would be even smaller than these calculations, if, for instance, CO_2 production were used to estimate flux through the DCA pathway (Merdink et al., 1998).

The estimated AUCL values for DCA following 1000 and 2000 mg/kg doses of TCE were 0.25 and 0.31 mg–hr/L if all non-TTC metabolites were DCA. The values were very similar for both doses due to the saturation of TCE metabolism coupled with its rapid exhalation during the

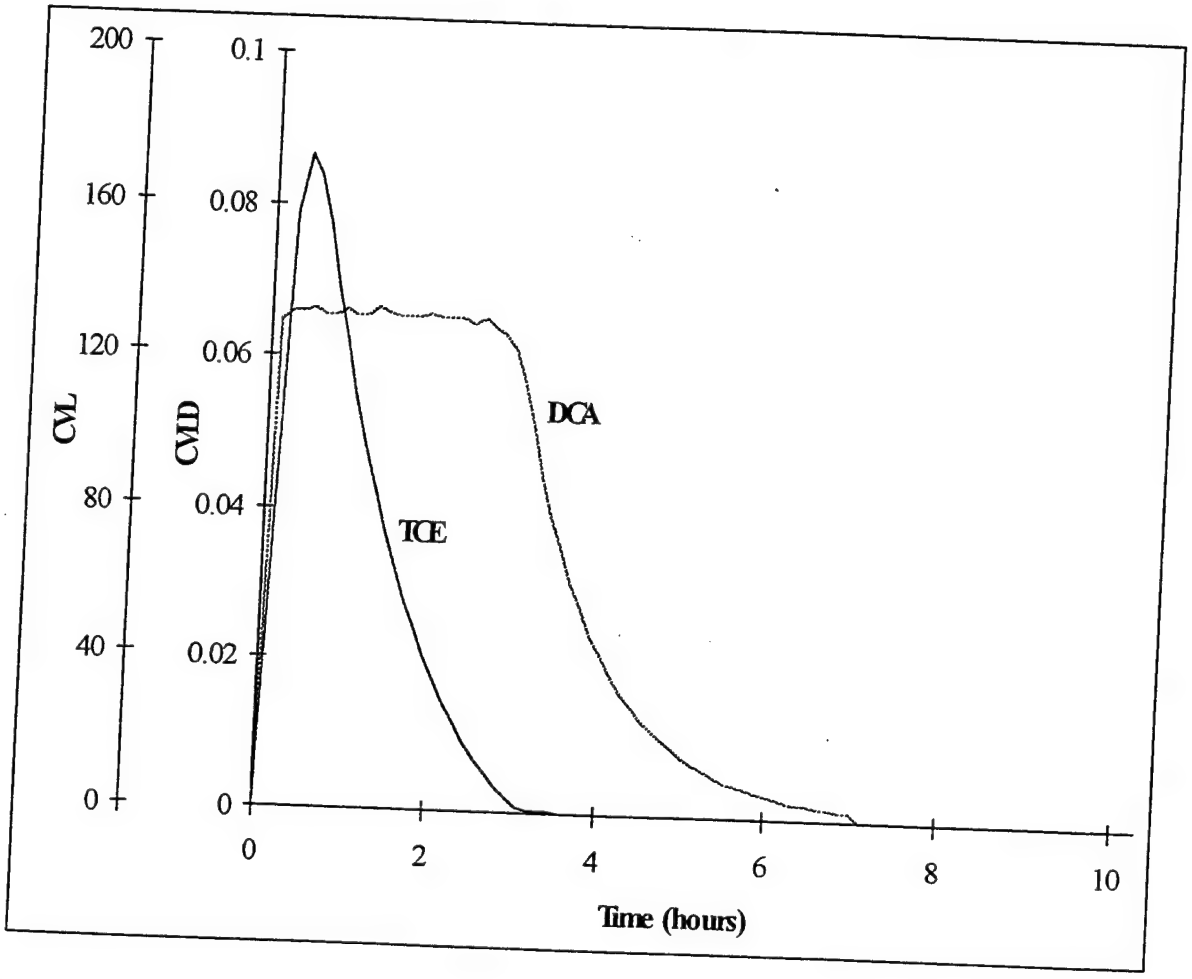

FIGURE 15: TCE and DCA in blood following oral gavage of 1000 mg/kg TCE. The solid line plots model simulations of blood concentrations of TCE (*cvl*) and DCA (*cvld*) following a single oral gavage dose of TCE.

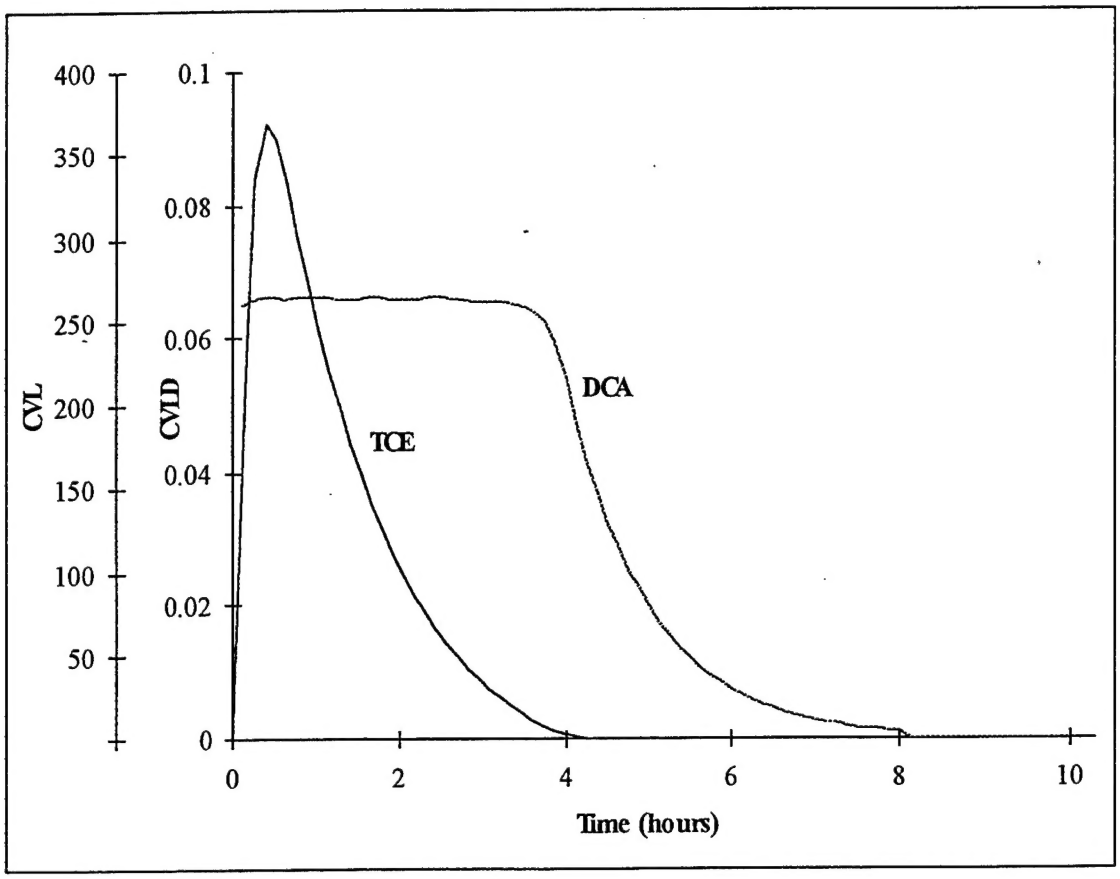

FIGURE 16: TCE and DCA in blood following oral gavage of 2000 mg/kg TCE. The solid line plots model simulations of blood concentrations of TCE (*cvl*) and DCA (*cvld*) following a single oral gavage dose of TCE.

period when metabolism is saturated. These values for liver exposure were between those estimated for the 0.05 g/L and 0.5 g/L drinking water exposures to DCA. The 0.05 g/L dose of DCA was not associated with an increase in tumor prevalence, though there was a small increase in multiplicity. By contrast, these doses of TCE were associated with large increases in liver cancer incidence in males and smaller, but significant increases in females. For example, in the NCI bioassay, males received approximately 1200 and 2400 mg/kg/day and females approximately 900 and 1700 mg/kg/day. Hepatocellular carcinomas were observed in 26/50 (52%) and 31/48 (65%) of males and 4/50 (8%) and 11/47 (23%) of females compared to 1/20 (5%) and 0/20 (0%) in male and female control mice, respectively.

CONCLUSIONS

A pharmacokinetic analysis for DCA in naive and DCA–pretreated mice has been conducted in order to interpret the chronic bioassay liver tumorigenicity results for DCA and the likelihood for the involvement of DCA in liver carcinogenicity induced by TCE. The kinetic data from control and pretreated mice following i.v. injection and oral dosing were critical for making this evaluation. These data confirm that there is a very large first pass metabolism of DCA in the liver and that the metabolism is significantly inhibited by exposure to high doses of DCA.

The chronic drinking water studies with DCA have largely been conducted at concentrations of 1 – 5 g/L. At these doses, inhibition of metabolism occurs relatively rapidly compared to the length of the bioassay. Thus, the large increases in tumor incidence that have been observed at these doses correlate with the increasing AUCL for the parent compound (DCA) that occurs as a consequence of saturation of its metabolism.

The analysis for TCE suggests that, if DCA is formed, the blood levels would be so small that increased analytical sensitivity would be necessary for their measurement. Because of the rapid metabolism of DCA, the AUCL for DCA would be very low following TCE exposure. The AUCL for DCA produced from oral TCE exposure is similar to that estimated for the 0.05 g/L DCA drinking water exposure which was not associated with an increase in the prevalence of liver cancer. Therefore it is highly unlikely that sufficient DCA will be present in the liver to contribute significantly to TCE-induced liver cancer.

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